

## MOLECULAR QUALITY ENGINEERING FOR LOW COST VACCINE PRODUCTION

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Vaccines based on recombinant proteins provide a compelling case for low cost products with broad global accessibility. Protein immunogens are typically derived directly from native sequences found in bacterial or viral pathogens, and may not be well-suited for efficient expression in recombinant hosts. Native immunogens may also suffer from numerous challenges during expression that impact their quality or efficient production, including truncation, aggregation and poor stability. These challenges can lead to inefficiencies in manufacturing of subunit protein vaccines.

Typically, recombinant vaccine manufacturing processes are complex, serial batch operations requiring extensive quality testing throughout to ensure product integrity. In response to the Gates Foundation's Grand Challenge for Innovations in Vaccine Manufacturing for Global Markets, we are co-developing the ULTRA program for flexible, low cost vaccine products. This program aims to develop platform processes for production of recombinant vaccines. We believe that molecular design of the antigens provides a critical handle in improving antigen quality, manufacturability, and product stability, all of which could enable potent, low-cost vaccines. Addressing potential manufacturing challenges early on in product development should enable simple integrated processes for antigen production while minimizing costs associated with quality testing.

To this end, we are demonstrating our platform approach with a recombinant trivalent subunit vaccine for rotavirus currently in clinical development. We chose to express the three VP8 subunits in *Pichia pastoris* to take advantage of the high titers of secreted proteins and minimal process-related contaminants typically experienced with this organism—critical features when developing simple intensified processes to meet our cost targets of \$0.15/dose. Initial expression results showed the rotavirus antigens were poorly expressed and suffered from N-terminal truncation and aggregation—all of which were also observed in a previously developed *E. coli*-based process. We have deployed a two-pronged approach toward improving the manufacturability of these antigens. First, we used a functional genomics approach to identify bottlenecks experienced during cellular expression of the antigens. RNA-sequencing is a mature, inexpensive and accessible technique for yeast that can indicate host- or sequence-derived bottlenecks in antigen transcription, translation and expression. Second, we made direct sequence changes to the antigens to mitigate specific quality challenges, such as aggregation. Iterations of this approach have enabled robust titers of rotavirus antigens with improved quality. This framework for incorporation of molecular engineering early in development provides a useful model for improving target product profiles that include manufacturability for low-costs, while maintaining immunogenicity.